

(12) PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 199874363 B2
(10) Patent No. 741964

(54) Title
Sustained-release compositions and method for preparing same

(51)⁶ International Patent Classification(s)
A61K 009/16 A61K 038/09

(21) Application No: 199874363 (22) Application Date: 1998.04.17

(87) WIPO No: WO98/47489

(30) Priority Data

(31) Number	(32) Date	(33) Country
97/04837	1997.04.18	FR
98/03666	1998.03.25	FR

(43) Publication Date : 1998.11.13
(43) Publication Journal Date : 1999.01.07
(44) Accepted Journal Date : 2001.12.13

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(56) Related Art
EP 709085
EP 579347
EP 384752

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OPI DATE 13/11/98 APPLN. ID 74363/98
AOJP DATE 07/01/99 PCT NUMBER PCT/FR98/00773



AU9874363

S (PCT)

(51) Classification internationale des brevets ⁶ : A61K 9/16, 38/09	A1	(11) Numéro de publication internationale: WO 98/47489 (43) Date de publication internationale: 29 octobre 1998 (29.10.98)
(21) Numéro de la demande internationale: PCT/FR98/00773 (22) Date de dépôt international: 17 avril 1998 (17.04.98) (30) Données relatives à la priorité: 97/04837 18 avril 1997 (18.04.97) FR 98/03666 25 mars 1998 (25.03.98) FR Ipsen Pharma Biotech (71) Déposant (pour tous les Etats désignés sauf US): PHARMA BIOPHARMA (FR/FR); Parc d'Activités du Plateau de Signes, Ch. Dep. No. 402, F-83870 Signes (FR). (72) Inventeurs; et (75) Inventeurs/Déposants (US seulement): PELLET, Marc (FR/FR); Bois de Fayot, F-27160 Condé sur Ilon (FR). ROUME, Chantal (FR/FR); Quartier de l'Hubac, 493, chemin des Beausitres, F-83870 Signes (FR). (74) Mandataire: BOURGOUIN, André; Société de Conseils Ad- ministratifs et Financiers (S.C.A.F.) - Service Brevets et Marques, 42, rue du Docteur Blanche, F-75016 Paris (FR).	(81) Etats désignés: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, brevet ARIPO (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), brevet eurasien (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), brevet européen (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), brevet OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Publiée <i>Avec rapport de recherche internationale. Avant l'expiration du délai prévu pour la modification des revendications, sera republiée si des modifications sont reçues.</i>	
(54) Title: SUSTAINED-RELEASE COMPOSITIONS AND METHOD FOR PREPARING SAME (54) Titre: COMPOSITIONS PRESENTANT UNE LIBERATION PROLONGEE ET LEUR PROCEDE DE PREPARATION		
(57) Abstract		
The invention concerns compositions in the form of microcapsules or implants comprising a biodegradable polymer or copolymer vehicle or mixture of vehicles with logarithmic viscosity number between 0.5 dl/g and 1.6 dl/g in CHCl ₃ and an active substance or mixture of active substances, said microcapsules or implants being capable of releasing the active substance or mixture of active substances over a prolonged period up to three months or more. These compositions can also comprise an active substance with high specific surface area.		
(57) Abrégés		
L'invention concerne des compositions sous forme de microcapsules ou d'implants comprenant un excipient ou un mélange d'excipients polymères ou copolymères biodégradables de viscosité inhérente comprise entre 0.5 dl/g et 1.6 dl/g dans CHCl ₃ et une substance active ou un mélange de substances actives, lesdits microcapsules ou implants pouvant libérer la substance active ou le mélange de substances actives sur une période prolongée pouvant aller jusqu'à trois mois ou plus. Ces compositions peuvent également comporter un principe actif présentant une surface spécifique élevée.		

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Sustained release compositions and the process for their preparation

The invention relates first of all to a composition in the form of microcapsules or implants comprising a biodegradable polymeric or copolymeric excipient or a mixture of such excipients with an inherent viscosity of between 0.5 dl/g and 1.6 dl/g in CHCl₃, and at least one active substance. The invention further relates to a composition in the form of microcapsules or implants comprising at least one biodegradable polymer or copolymer of high molecular weight and at least one water-soluble active substance of high specific surface area. Such compositions will be used to obtain a uniform release of the active substance over a prolonged period of up to more than three months.

These compositions, and especially the microcapsules, are mainly used in pharmacy, but can also be employed in other sectors, particularly in agrochemistry, i.e. in the plant protection sector.

The value of administering active principles in the form of sustained release compositions has been known for a long time, whether they be conventional pharmaceutical products, for example steroids, peptides or proteins (cf., for example, Patent US 3,773,919 to Boswell), or products for use in plant protection. The formulations adopted can take the form of microparticles in which the active principle is incorporated in a biodegradable polymer or copolymer such as a polylactide/co-glycolide copolymer (PLGA).

It has been found that, especially when a relatively constant or, in any case, uninterrupted release mode is sought - this mode being referred to for example as "monophase" in European Patent EP 58 481 - PLGA-type polymers of relatively low molecular weight, i.e. of low viscosity, are required. European patents EP 21 234 (cf. Example 8.B.2. describing a copolymer of intrinsic viscosity 0.5 dl/g), EP 52 510, in which a copolymer with a viscosity of 0.38 dl/g in hexafluoroisopropanol (HFIP) is tested *in vivo*, and EP 26 599, which describes, by way of example, polymers with viscosities of 0.12 to 0.20 dl/g and claims polymers with a viscosity of 0.08 to 0.30 dl/g, may be mentioned in this connection. The polymers described in said patents are presented as producing constant release compositions. The compositions of Patent EP 26 599 can contain fertility control agents, for example.



It is moreover important to note in this respect that in the opposition proceedings relating to European Patent EP 58 481, which are still in progress on the date of filing of the present patent application, the Applicant limited its main claim to polymers of low viscosity (below 0.3 or 0.5 dl/g), which, according to the Applicant,
5 are the only ones capable of permitting a release of the monophasic type.

Furthermore, when a longer release period, for example of more than one month, is sought, more complex problems appear and a solution proposed by Patent EP 0 302 582, for example, consists in mixing several types of microcapsules made up of polymers of different viscosities.

10 Now, the present Applicant has just found that certain polymers of high viscosity are suitable for the preparation of long-term sustained release compositions. It has also been found that the use of certain polymers produces compositions which have a very long-term monophasic release profile without an initial period of no release (dead period). This applies particularly to polymers with an inherent viscosity
15 preferably of at least 0.5 dl/g in CHCl_3 and more preferably of at least 0.6 or 0.7 dl/g. In principle, however, the inherent viscosity of these polymers will not exceed 1.6 dl/g in CHCl_3 and may be below 1.4 or 1.2 dl/g. Said polymers will preferably be PLGAs with a lactide/glycolide ratio varying from 40/60 to 90/10, and being preferably about 75/25.

20 The polymers according to the invention can be prepared by the customary methods, especially by opening of the lactide or glycolide rings. Such a process is described for example in American Patent US 3,773,919.

In the present invention it is also possible to use a mixture of polymers of different high viscosities, but compositions containing only one polymer or copolymer are
25 preferred.

The invention therefore relates first of all to a composition in the form of microcapsules or implants comprising a biodegradable polymeric or copolymeric excipient or a mixture of such excipients with an inherent viscosity of between 0.5 dl/g and 1.6 dl/g in CHCl_3 , and an active substance or a mixture of active substances,
30 it being possible for these microcapsules or implants to release the active substance



or mixture of active substances over a prolonged period of at least 1 month, preferably of at least 2 months and more preferably of at least 3 months.

Microcapsule is also understood to include microspheres, microparticles, nanocapsules, nanospheres or nanoparticles. Polymer will be understood as meaning a
5 polymer, a copolymer or any mixture of these entities. Finally, active substance is understood as meaning an active substance, one of its salts, one of its precursors or any mixture of these compounds.

Salts of active substances which can be used for compositions according to the invention include especially the salts obtained from organic acids like acetic, malic,
10 tartaric, oxalic, fumaric, citric, lactic, stearic, pamoic, methanesulphonic or p-toluenesulphonic acids, or from inorganic acids like hydrochloric, sulphuric, phosphoric or hydrobromic acids. It will be preferable to use a water-soluble product obtained by salification in the form of a cation, for example with acetic acid. However, it is possible to use an insoluble salt, for example a pamoate.

15 Particularly, the invention relates to a composition in the form of microcapsules or implants comprising a biodegradable polymeric or copolymeric excipient or a mixture of such excipients and an active substance or a mixture of active substances, said microcapsules or said implants being able to release the active substance or the mixture of active substances over a prolonged period of time of up to three months or
20 more with an essentially monophasic release profile, said composition being characterized in that :

- either, when the composition is in the form of microcapsules:

- either the viscosity of said polymers or copolymers is comprised between 0.7 dl/g and 1.6 dl/g in CHCl_3 and the preparation process for said
25 microcapsules does not comprise any stage of fusion of said microcapsules,
- or the viscosity of said polymers or copolymers is comprised between 0.5 dl/g and 1.6 dl/g in CHCl_3 and said polymers or copolymers have an hydrophilic character ;

- or, when the composition is in the form of implants, the viscosity of said polymers
30 or copolymers is comprised between 0.5 dl/g and 1.6 dl/g in CHCl_3 .

Preferably, the viscosity of the polymers or copolymers for the compositions according to the invention will be at least equal to 0.9 dl/g in CHCl_3 .

The polymers or copolymers which can be used for the invention can be especially polymers such as those of lactic acid, glycolic acid, citric acid or malic acid, or else



5 other biocompatible polymers like poly- β -hydroxybutyric acid, polyorthoesters, polyorthocarbonates, poly- α -cyanoacrylic acid esters, polyalkylene oxalates such as polytrimethylene or polytetramethylene oxalate, polyamino acids, etc. They can also be copolymers like PLGA, polystyrene, polymethacrylic acid, methacrylic
10 acid/acrylic acid copolymers, polyamino acids, maleic anhydride polymers, ethyl cellulose, nitrocellulose, acetyl cellulose, etc. All these polymers or copolymers can be used by themselves or in any mixture. Generally, the PLGAs will comprise from 40 to 90 % of lactide and from 10 to 60 % of glycolide. It will be preferable to use D,L-PLGA and more preferable to use a D,L-PLGA produced from 70 to 80% of
15 DL-lactide and 20 to 30% of glycolide. A PLGA synthesized from 75% of DL-lactide and 25% of glycolide will be particularly suitable for the invention.

Another particularly preferred polymer for the invention is L-PLGA obtained from L-lactide and glycolide. Compared with D,L-PLGA of the same viscosity, L-PLGA assures a slower release and represents an alternative to the D,L-PLGAs of higher
20 viscosity.

In a general manner, the polymers or copolymers possessing a hydrophilic character will be preferred. Hence, preference will generally be given to the PLGAs obtained by ring opening with hydrophobic initiators such as those of the lauryl alcohol type, those obtained by ring opening with hydrophilic initiators such as those of the lactic
25 acid or glycolic acid type.

By polymer or copolymer possessing a hydrophilic character, it is meant a polymer or copolymer for which the terminal chain is polar (for example, this terminal chain includes at its end an acidic function), by opposition to a polymer or copolymer possessing a hydrophobic character for which the terminal chain is apolar (for
30 example, this terminal chain is an aliphatic chain).

The acid number, which corresponds to the number of milliequivalents of KOH required per gram of polymer to neutralize the free acidity, seems to be the parameter which correlates best with the hydrophilic or hydrophobic character of a polymer or copolymer. Wherever the terminal chains of the polymers or copolymers may include
35 a free acidic function, owing to the nature of the monomer, this acid number may be measured.



In a general manner, the Applicant has found that the hydrophilic polymers produce a better release profile. Hence, the acid number of the polymers used for the invention will preferably be at least equal to 1, or better, 1.2, and more preferably at least equal to 1.5 or 2.

- 5 The core loading of the microcapsules according to the invention, i.e. the ratio of the weight of encapsulated pure peptide to the total weight of the microcapsule, will generally be between 0 and 20% and preferably between 2 and 15%. In the case of triptorelin acetate, the loading will preferably be less than or equal to 10% and more preferably between 4 and 8% for forms which permit release over a period of about 3
10 months. In the case of lanreotide acetate, the loading will preferably be between 10 and 20%.

In the case of implants, the core loading will generally be between 0 and 30% and preferably between 15 and 25%.

- The encapsulation step can be a so-called coacervation step, such as that described in
15 American Patent US 3,773,919 or European Patent EP 52 510.

It is also possible to use a so-called melting-extrusion process such as that described in European Patent EP 58 481 or American Patent US 5,225,205, the products obtained then optionally being ground by the customary methods to give microparticles.

- 20 In another respect, a water-soluble active principle such as a water-soluble salt of a peptide, for example the acetate, can be used. It is also possible to use an insoluble salt of a soluble molecule, such as a fatty acid salt of a peptide, for example a peptide pamoate such as that described in British Patent GB 2 209 937.

- The compositions obtained by melting-extrusion using the polymers according to the
25 invention can also be presented in the form of implants and be used as such.

These implants are preferably small (mini-implants or microimplants) with a diameter of the order of 1 mm, for example of between 0.8 and 1.2 mm. The length of these implants can be for example between 10 and 35 mm, for example of the order of 25 mm. These implants give very advantageous results with low doses of



active principle, for example of the order of 3 mg of triptorelin acetate per implant. Such implants can release the active principle over a period of up to 3 months.

Moreover, it has been found that the form of the active principle can also influence the diffusion of this product. In particular, if an active principle can be obtained in a
5 crystalline or amorphous form, it is not arbitrary which of the two forms is chosen.

Patent application EP 709 085 describes microcapsules comprising a polymer and an amorphous water-soluble active substance. It is particularly concerned with the importance of obtaining small particles of active substance, preferably with a size of less than 10 μm . However, this patent application does not disclose any process for
10 the preparation of said particles and no mention is made of the effect of the specific surface area of the particles of active principle on the release profile of the compositions containing these particles. Now, the Applicant has already been using, since 1986, microcapsules containing an amorphous active substance, namely triptorelin acetate, sold under the name Decapeptyl 3.75 mg, which has a particle size
15 of only about 8 μm . However, it has found that the particle size is not the only determining parameter for favouring release over a prolonged period of up to more than three months or more.

In principle, the question of amorphous character does not arise for products such as peptides or proteins, whose method of preparation, especially lyophilization, leads to
20 an amorphous product in the majority of cases, as for Decapeptyl 3.75 mg.

There are copious illustrations of this phenomenon in the literature and the following articles may be mentioned in particular: Hsu, C.C. et al., *Pharmaceutical Research*, 12 (1), 69 - 77 (1995) or Towns, J.K., *Journal of Chromatography*, A, 705 (1), 115 - 27 (1995).

25 The invention therefore also relates to a composition in the form of microcapsules or implants comprising at least one biodegradable polymer or copolymer of high molecular weight and at least one water-soluble active substance of high specific surface area. Particularly, said specific surface area is greater than 2 m^2/g , and preferably greater than 3 m^2/g . More preferably, said specific surface area is greater
30 than 5 m^2/g or 10 m^2/g . Even more preferably, said specific surface area is greater than 20 m^2/g , and preferably greater than 30 m^2/g .



The invention relates preferably to the above compositions in which the water-soluble active substance is a protein or a peptide.

It further relates to the above compositions for which the polymer or copolymer viscosity is comprised between 0.5 and 1.6 dl/g in CHCl_3 , and preferably comprised
5 between 0.9 and 1.6 dl/g in CHCl_3 . Particularly, polymers or copolymers of viscosity comprised between 0.7 and 1.3 dl/g in CHCl_3 may be chosen and more preferably polymers or copolymers of viscosity comprised between 0.7 and 1.3 dl/g. PLGAs are particularly adapted for the invention. Preferably, said PLGAs will be produced from
10 40 to 90 % lactide and 10 to 60 % glycolide, and more preferably from 70 to 80 % lactide and 20 to 30 % glycolide. The hydrosoluble active substances incorporated in the microcapsules or implants will preferably be proteins or peptides.

Compositions comprising an active substance of high specific surface area will preferably be such that viscosity of the polymer or copolymer is comprised between
15 0.5 and 1.6 dl/g in CHCl_3 and that the polymer or copolymer presents an hydrophilic character, the acid number of the latter being greater than 1 meq KOH per gram of polymer or copolymer, and preferably greater than 1.2, more preferably 1.5 mEq or even 2 meq KOH per gram of polymer or copolymer.

The invention further relates to compositions in the form of microcapsules or
20 implants comprising an active substance of high specific surface area characterized in that the polymer or copolymer is a PLGA, and preferably a PLGA produced from 70 to 80 % lactide and 20 to 30 % glycolide, the viscosity of said PLGA being comprised between 0.5 and 1.6 dl/g on CHCl_3 and the active substance incorporated in the microparticles or implants being a protein or a peptide.

25 These microcapsules or implants permit a monophasic release profile in which the initial peak (or burst) is reduced in comparison with certain other preparations using a polymer of lower molecular weight, so they make it possible to release the active substance over a prolonged period of up than three months or more.

In other words, the Applicant has found that the release properties, notably the
30 monophasic type release, of compositions in the form of microcapsules or implants, particularly of compositions based on PLGA and including as an active principle a peptide or a protein, are considerably improved if at least one of the following characteristics is present :



- a) the polymer or copolymer is a PLGA which presents a viscosity in chloroform of at least 0.5 dl/g, preferably of at least 0.9 dl/g and in principle lower than 1.6 dl/g ;
- b) the polymer or copolymer is a PLGA which is prepared from 70 to 80 % lactide and from 20 to 30 % glycolide ;
- 5 c) the polymer or copolymer presents an hydrophilic character, and preferably has an acid number greater than 1 mEq KOH, and more preferably greater than 1.2 or even 1.5 mEq KOH per gram of polymer or copolymer ;
- d) the active principle, preferably a peptide or a protein, has a high specific surface area and greater than 2 m²/g, preferably greater than 10 m²/g, more preferably greater than 20 m²/g or even greater than 30 m²/g ;
- 10

these characteristics being optionally combined with the use of a L-PLGA instead of a D,L-PLGA.

- 15 According to its present knowledge, the Applicant is of the opinion that characteristic d) taken alone is very important and may be advantageously combined to the other characteristics a) b) or c). Particularly, the characteristic d) may be combined to following characteristics : a) alone, b) alone, c) alone, a) and b) together, a) and c) together, b) and c) together, or a), b) and c) together. More preferably, characteristic d) will be combined at least with characteristic c).
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Among the active substances which can be used for the different aspects of the invention, there may be mentioned in particular proteins and peptides. Said active substances can be selected for example from the group consisting of the following substances: triptorelin or one of its salts, particularly triptorelin acetate, lanreotide or one of its salts, particularly lanreotide acetate, octreotide or one of its salts (as described for example in European Patent EP 29 579), particularly octreotide acetate or pamoate, a compound with LH-RH activity, such as triptorelin, goserelin, leuprorelin, buserelin or their salts, an LH-RH antagonist, a GPIIb/IIIa antagonist, a compound with a similar activity to a GPIIb/IIIa antagonist, erythropoietin (EPO) or one of its analogues, the various types of interferon- α , interferon- β or - γ , somatostatin, a somatostatin derivative such as that described in European Patent EP 215 171, a somatostatin analogue such as that described in American Patent US 5,552,520 (this patent itself includes a list of other patents describing somatostatin analogues, which are incorporated in the present patent application by way of reference), insulin, a growth hormone, a growth hormone releasing factor

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(GHRF), a growth hormone releasing peptide (GHRP), an epidermal growth factor (EGF), a melanocyte stimulating hormone (MSH), a thyrotropin releasing hormone (TRH) or one of its salts or derivatives, a thyroid stimulating hormone (TSH), a luteinizing hormone (LH), a follicle stimulating hormone (FSH), a parathyroid hormone (PTH) or one of its derivatives, a lysozyme hydrochloride, a parathyroid hormone related peptide (PTHrp), an N-terminal peptide fragment (position 1→34) of human PTH hormone, vasopressin or one of its derivatives, oxytocin, calcitonin, a calcitonin derivative with a similar activity to that of calcitonin, a calcitonin gene related peptide (CGRP), glucagon, a peptide similar to glucagon (GLP), gastrin, a gastrin releasing peptide (GRP), secretin, pancreaticozym, cholecystokinin, angiotensin, human placental lactogen, human chorionic gonadotropin (HCG), enkephalin, an enkephalin derivative, colony stimulating factor (CSF), endorphin, kyotorphin, interleukins, for example interleukin-2, tuftsin, thymopietin, thymosthymin, thymic humoral factor (THF), thymic serum factor (TSF), a derivative of thymic serum factor (TSF), thymosin, thymic factor X, tumour necrosis factor (TNF), motilin, bombesin or one of its derivatives as described in American Patent US 5,552,520 (this patent itself includes a list of other patents describing bombesin derivatives, which are incorporated in the present patent application by way of reference), prolactin, neurotensin, dynorphin, caerulein, substance P, urokinase, asparaginase, bradykinin, kallikrein, nerve growth factor, a blood clotting factor, polymixin B, colistin, gramicidin, bacitracin, a protein synthesis stimulating peptide, an endothelin antagonist or one of its salts or derivatives, a vasoactive intestinal polypeptide (VIP), adrenocorticotrophic hormone (ACTH) or one of its fragments, a platelet derived growth factor (PDGF), a bone morphogenetic protein (BMP), a pituitary adenylate cyclase activating polypeptide (PACAP), neuropeptide Y (NPY), peptide YY (PYY), a gastric inhibitory polypeptide (GIP) and polynucleotides, especially double-stranded RNAs (ds-RNAs) such as those described in Patent application EP 0 300 680 or French Patent no. 2 622 586.

ds-RNA is preferably understood as meaning polyadenylic acid complexed with polyuridylic acid, which is also called poly(A)-poly(U) or Poly-adenur[®]. Other ds-RNAs can be used for the invention, especially a complex of polyinosinic acid with polycytidylic acid, which is also known by the name poly(I)-poly(C), as well as these same complexes modified by the introduction of uridylic acid into the polycytidylic acid chain, such as the product Ampligen[®] from the HEMISPHERx company (for a description of these products, reference may be made especially to European Patent application EP 0 300 680). The ds-RNA used can be for example a mixture of ds-



RNAs as defined above. The ds-RNAs are preferably prepared by the process described in French Patent no. 2 622 586.

5 A high specific surface area can be obtained for the previously mentioned substances as soon as they are water-soluble or transformed into water-soluble substances, for example by salification or grafting of a water-soluble chain on their structure. This is particularly valid for the previously mentioned peptides and proteins. Any other water-soluble active substance or one of its salts or precursors, and particularly the salts obtained by salification with acetic acid, may also be used by a person skilled in the art for this aspect of the invention if they consider it appropriate.

10 According to one of the preferred aspects of the invention, the peptide or protein with a high specific surface area are chosen from the group consisting of triptorelin acetate, lanreotide acetate or octreotide acetate.

15 Peptide and/or protein are understood in the present application as meaning both the peptide and/or the protein themselves and pharmacologically active fragments, salts or derivatives of these peptides or proteins.

The water-soluble active substance as used to manufacture microcapsules or implants according to the invention, and particularly triptorelin acetate, lanreotide acetate, octreotide acetate, goserelin, leuprorelin, buserelin or their salts, is preferably obtained by a process which principally involves two steps:

20 - a lyophilization step comprising the rapid immersion of a dilute solution of the water-soluble substance in a medium whose temperature is below -50°C , and preferably below -70°C ; and

- optionally a grinding step, which will preferably comprise ultrasonic grinding.

25 Dilute solution of the active substance is understood as meaning a solution whose concentration of said active substance is less than half the saturation concentration and preferably less than a quarter of said saturation concentration when the latter is at least equal to at least 200 g/l. This process produces an active substance of high specific surface area.



Rapid immersion must be understood as meaning contact with a low temperature medium, causing instantaneous freezing of the solution of water-soluble substance.

For the lyophilization, the solution may be frozen for example in a tray floating in a tank of liquid nitrogen, before the actual lyophilization is carried out.

5 Preferably, in order to obtain a maximum specific surface area, the rapid immersion of the solution will be preceded by a micronization of the solution of active substance. When the solution of active substance is micronized beforehand, the temperature of the low temperature medium may only be below -50°C .

10 For example, to obtain a very high specific surface area, it may be chosen to atomize the solution by spraying it through an atomizer onto a metal plate at very low temperature. The temperature of the plate will preferably be below -50°C and more preferably below -70°C or even -80°C or -120°C . This temperature may be reached for example by immersing a metal plate in a very low temperature medium, for example liquid nitrogen. According to one preferred variant of the invention, the
15 metal plate is hollow and the solution is sprayed inside said plate by means of an atomizer.

20 Other freezing techniques can be considered, for example atomization of the solution of active substance into a precooled bath of a non-solvent for said active substance. The non-solvent will preferably be a liquefied gas, such as for example liquid nitrogen.



Another possibility is to freeze the active substance solution on a rotating plate (drum-freezing). As previously indicated, this freezing will preferably be preceded by a micronization of the active substance solution.

5 When the process of freezing in a tray is applied to an active substance in order to prepare sustained release microcapsules or implants according to the invention, the specific surface area of the active substance, after lyophilization but before grinding, will preferably be greater than $2 \text{ m}^2/\text{g}$. The specific surface area of the active substance will more preferably be greater than $3 \text{ m}^2/\text{g}$ or even $5 \text{ m}^2/\text{g}$.

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If a specific surface area greater than $10 \text{ m}^2/\text{g}$ is required, the process which includes a micronization step will preferably be employed. The specific surface area obtained for the active substance after lyophilization will preferably be greater than $15 \text{ m}^2/\text{g}$. This specific surface area will even more preferably be greater than $20 \text{ m}^2/\text{g}$ or even

15 $30 \text{ m}^2/\text{g}$.

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The specific surface areas obtained may be varied by varying the freezing conditions of the solution of active substance by way of different parameters, such as for example the freezing rate or the concentration of the solution.

5 The specific surface area of the active substance is a favourable factor for obtaining release over a prolonged period, particularly in the case of microcapsules. In fact, as already mentioned, particles of an active substance which have the same size but different specific surface areas will give totally different results with the same polymeric excipient.

10 The invention therefore also relates to the processes as described above, applied to a biologically active water-soluble substance. It further relates to the biologically active water-soluble substance as obtained by these processes, said substance having a high specific surface area.

15 Particularly, the invention relates to triptorelin acetate, lanreotide acetate or octreotide acetate as obtained by the previously described processes, or to a double strand RNA, preferably polyadenylic acid complexed with polyuridylic acid as obtained by these processes.

20 As indicated above, the compositions according to the invention are preferably used in the pharmaceutical sector. The pharmaceutical compositions can be administered to a patient by different routes, however, the preferred route is subcutaneous or intramuscular injection. The microcapsules according to the invention can first be suspended in a vehicle appropriate for injection, such as an aqueous solution of sodium chloride or an aqueous solution of mannitol.

25 Unless defined otherwise, all the technical and scientific terms used here have the same meanings as those commonly understood by an ordinary specialist in the field to which this invention belongs. Likewise, all the publications, patent applications, patents and any other references mentioned here are incorporated by way of reference.

30 The following examples are presented in order to illustrate the above procedures and must not under any circumstances be considered as limiting the scope of the invention.



EXAMPLES:

For all these examples, the inherent viscosities (IV) were measured by the conventional methods of flow time measurement, as described for example in "Pharmacopée Européenne", 1997, pages 17 - 18 (capillary tube method). Unless
5 stated otherwise, these viscosities have been measured in chloroform at a concentration of 0.1% at 25°C or in hexaisofluoropropanol at a concentration of 0.5% at 30°C. Where measured, the specific surface area of the active substance was determined by the so-called BET method (absorption of a nitrogen monolayer on the active substance), a method well known to a person skilled in the art.

10 For the following examples, a peptide which has undergone the lyophilization process according to the invention will be called the "modified" peptide, in contrast to the "unmodified" peptide, which is lyophilized in conventional manner (without sudden immersion at low temperature).

Example 1:

15 16.620 g of "unmodified" triptorelin acetate are dissolved in 554 ml of water. The solution is frozen in a tray floating in a tank of liquid nitrogen, then lyophilized.

15.18 g of "modified" triptorelin acetate are thus obtained with a yield of 91.34%. This compound has a specific surface area of 4.7 m²/g, compared with 0.8 m²/g before lyophilization.

20 The triptorelin acetate is then subjected to ultrasonic grinding: 15 minutes are sufficient to obtain particles smaller than 10 µm with the modified peptide (whereas 30 minutes are required to obtain this particle size with the unmodified peptide).

The encapsulation step is then performed by the coacervation method as described in European Patent EP 52 510 and American Patent US 3,773,919, starting from
25 3.378 g of this ground modified triptorelin acetate and a 7.30% solution of D,L-PLGA (D,L-PLGA composed of 75% of DL-lactide and 25% of glycolide, inherent viscosity in chloroform = 0.70 dl/g, acid number = 1.61 meq KOH/g) in dichloromethane. 390 ml of silicone oil were added in order to form microcapsules by the coacervation process. These microcapsules are recovered after immersion in a
30 bath of heptane (22 l) and filtration on a 10 µm membrane.



Example 2:

0.338 g of unmodified triptorelin acetate, with a particle size of 8 μm after ultrasonic grinding for 30 minutes, was added, with stirring, to a 7.30% solution of D,L-PLGA in dichloromethane (PLGA equivalent to that described in Example 1). 40 ml of silicone oil were added in order to form microcapsules, which were subsequently precipitated in a bath of heptane (2 l) and then filtered on a 10 μm membrane.

Examples 3 to 6:

0.338 g of triptorelin acetate modified under the conditions described in Table no. 1 below was added, after ultrasonic grinding, to a 7.30% solution of a 33.3%/33.3%/33.3% mixture of three D,L-PLGAs (having the characteristics described in Table no. 2 below) in dichloromethane. 40 ml of silicone oil were added in order to form microcapsules, which were subsequently precipitated in a bath of heptane (2 l) and then filtered on a 10 μm membrane.

Table no. 1

15

Example	Concentration (g/l)	Amount of triptorelin acetate (g)	Amount of water (ml)	Specific surface area (m^2/g)
3	200	3	15	4.4
4	150	3	20	4.7
5	100	3	30	4.8
6	50	3	60	7.3

The specific surface area of the (unmodified) starting triptorelin acetate is 0.8 m^2/g .

The physicochemical characteristics of the three mixed polymers are collated in Table no. 2 below:



Table no. 2

Characteristic	PLGA no. 1	PLGA no. 2	PLGA no. 3
Lactide/glycolide ratio	D,L-PLGA 50:50	D,L-PLGA 75:25	D,L-PLGA 75:25
Inherent viscosity in CHCl ₃ (dl/g)	0.47	0.61	0.70
Acid number (meq KOH/g)	2.68	2.08	1.61

Example 7:

22.560 g of unmodified lanreotide acetate are dissolved in 752 ml of water. The solution is frozen in a tray floating in a bath of liquid nitrogen, and then lyophilized.
5 21.75 g of modified lanreotide acetate with a specific surface area equal to 4.4 m²/g are obtained with a yield of 96.41%.

The encapsulation step is then performed by the coacervation method as described in European Patent EP 52 510 and American Patent US 3,773,919, starting from 7.5 g of this ground modified triptorelin acetate and a 3.7% solution of D,L-PLGA
10 (D,L-PLGA composed of 50% of DL-lactide and 50% of glycolide, inherent viscosity in HFIP = 0.55 dl/g) in dichloromethane. 650 ml of silicone oil were added in order to form microcapsules by the coacervation process. These microcapsules are recovered after immersion in a bath of heptane (30 l) and filtration on a 10 µm membrane.

15 Examples 8 and 9:

Microcapsules of triptorelin acetate were manufactured with D,L-PLGA (D,L-PLGA composed of 75% of DL-lactide and 25% of glycolide) of different weight average molecular weights (Mw). They were manufactured by the process described in Example 1 using a triptorelin-acetate with a specific surface area of 4.7 m²/g.

20 The physicochemical parameters of Examples 8 and 9 are collated in the table below:



Example	Mw THF	IV CHCl ₃ (dl/g)	Acid number (meq KOH/g)
8	58,400	0.61	2.08
9	132,650	0.93	1.31

Example 10:

Microcapsules were manufactured according to the process described in Example 1 using D,L-PLGA (D,L-PLGA composed of 75% of DL-lactide and 25% of glycolide; molecular weight determined in THF: 80,100; viscosity in chloroform: 0.75 dl/g; acid number = 0.40 meq KOH/g) having a hydrophobic tendency.

Example 11:

Microcapsules were manufactured according to the process described in Example 1, starting from an L-PLGA (L-PLGA composed of 75% of L-lactide and 25% of glycolide; molecular weight in THF: 99,260; viscosity in chloroform: 0.78 dl/g; acid number = 1.80 meq KOH/g) having a crystalline tendency.

Example 12:

One part by weight of triptorelin acetate is added to four parts by weight of powdered D,L-PLGA (PLGA composed of 75% of lactide and 25% of glycolide; molecular weight determined in THF: 103,810; inherent viscosity in chloroform: 0.82 dl/g).

The lumps are destroyed by sieving on a 400 μ m mesh, the product is mixed for 20 minutes at 42 rpm and the mixture is extruded at 120°C through a die of diameter 1 mm on a screw extruder. The extrudate is then cooled in air and sized by drawing (drawing device) to a final diameter of 0.85 mm.

The concentration of the mixture per unit length (mm) is determined and the rods of extrudate are cut to calculated lengths (in this case 24 mm) so that the microimplants contain a 3 mg dose of triptorelin. Finally, the weight of each microimplant is checked.

Examples 13 and 14:



The same protocol is used for these two examples:

5 g of lanreotide acetate are dissolved in water in order to give the solution the chosen concentration (for example, to obtain a concentration of 30 g/l, 167 ml of sterile water are added). This solution is atomized with a 500 ml sprayer whose jet is adjusted so as to give the finest possible droplets. The droplets obtained are sprayed into a tray, the bottom of which is immersed in liquid nitrogen. Two temperature probes are introduced into the tray beforehand so that the change in the temperature of the product can be monitored.

10 Once the product is frozen, the tray is introduced into a lyophilizer whose plate is at about -54°C .

The temperature of the products and that of the plate are left to equilibrate for 1 hour. This leads on to the sublimation stage (the temperature of the plate is set at 20°C and the pressure in the tank at $100\ \mu\text{bar}$). This stage lasts about 30 hours. The mean final temperature of the product is 13°C . The secondary desiccation which follows (pressure of $50\ \mu\text{bar}$ in the tank) lasts about 24 hours. The mean final temperature of the product is 20°C .

The characteristics of the reactants used and the products obtained are summarized in the table below:

Characteristics	Example 13	Example 14
Weight of lanreotide acetate used (g)	5.00	5.00
Concentration of the solution (g/l)	30	10
Weight of lanreotide acetate recovered (g)	4.54	4.10
Specific surface area obtained (m^2/g)	36	43

20 The lanreotide acetate of specific surface area $43\ \text{m}^2/\text{g}$ obtained previously (Example 14) is incorporated into microcapsules according to the following process:

0.782 g of lanreotide acetate is weighed into a glass tube. 15 ml of dichloromethane are added to the peptide salt. The peptide is subjected to ultrasonic grinding by means of an ultrasonic generator equipped with an amplifier and a dipping or flat ended probe (frequency = 50 Hz, power = 250 W; grinding lasts about 15 min).



The encapsulation step is then performed according to the coacervation method as described in European Patent EP 52 510 and American Patent US 3,773,919, using the 0.782 g of ground lanreotide acetate and a solution of 4 g of 50:50 D,L-PLGA (TV = 0.48 dl/g in CHCl₃) in 35 ml of dichloromethane. 34.2 ml of silicone oil were added in order to form microcapsules by the coacervation process. These microcapsules are recovered after immersion in a bath of heptane (2.5 l) and filtration on a 10 µm membrane.

The microspheres obtained can then be dried under vacuum, divided up into bottles and lyophilized with excipients (for example ballast or a surfactant) to enable storage under good conditions and to facilitate suspension of the microcapsules.

Examples 15 and 16:

A protocol similar to the one of Example 1 is used for these two examples. The peptide used is the same as that of these examples. The characteristics in terms of used PLGA, amount of peptide used (for these examples, modified triptorelin acetate) and preparation parameters of the microcapsules are listed in the table below:

Characteristics	Example 15	Example 16
Weight of modified triptorelin acetate used (g)	2.58	2.58
Quantity of polymer used (g)	30	30
Quantity of silicone oil used (ml)	600	650
Quantity of dichloromethane used (ml)	812	812
Lactide / glycolide ratio	75 / 25	75 / 25
Viscosity of the PLGA in CHCl ₃ (dl/g)	0.93	0.96
Measured acid number	1.22	1.12



Study of the release profiles of microcapsules according to the invention:

In order to illustrate the value of microcapsules according to the invention, their release profiles were studied *in vitro*.

For each of Examples 1 to 11 and 15 to 16, the release from three samples of about
5 25 mg of microcapsules (about 20 mg for Example 7), placed in 4 ml of 0.9% sodium chloride solution, is measured. Extraction is carried out after 1 hour, 1 day and 4 days of release into the solution, maintained at 37°C.

The triptorelin is determined by high performance liquid chromatography (HPLC),
10 relative to a calibration range, in gradient mode in a trifluoroacetic acid (TFA) system. To obtain the standard calibration range for triptorelin, a solution T₁ is prepared as follows: a sample of about 7.5 mg of reference triptorelin acetate is placed in a 50 ml flask; it is made up to 50 ml with 0.1% acetic acid solution. Solutions T₂ and T₃ are prepared from solution T₁ as follows: for T₂, 10 ml of
15 solution T₁ are taken and made up to 20 ml with 0.1% acetic acid solution. For solution T₃, 1 ml of solution T₁ is taken and made up to 50 ml with 0.1% acetic acid solution.

The lanreotide is determined in a similar fashion by HPLC. To obtain the standard calibration range for lanreotide, a solution T₁ is prepared as follows: a sample of about 16.5 mg of reference triptorelin acetate is placed in a 50 ml flask; it is made up to 50 ml with 0.1% acetic acid solution. Solutions T₂, T₃, T₄ and T₅ are prepared from solution T₁ as follows: for T₂, 10 ml of solution T₁ are taken and made up to 25 ml with 0.1% acetic acid solution. For solution T₃, 5 ml of solution T₁ are taken and made up to 25 ml with 0.1% acetic acid solution. Solution T₄ is obtained by dilution of 2 ml of solution T₁ in a 0.1% acetic acid solution in order to obtain a total volume of 25 ml, and solution T₅ by dilution of 1 ml of solution T₁ in a 0.1% acetic acid solution in order to obtain a total volume of 25 ml.

The amount of triptorelin acetate or lanreotide acetate released is determined as a percentage relative to the amount of triptorelin acetate or lanreotide acetate initially
20 present (100%), which serves as the reference.

The results of the *in vitro* tests are summarized in the table below:



Examples	Cumulative amount released (%)		
	1 hour	1 day	4 days
1	8	16	27
2	9	47	57
3	10	45	67
4	10	37	65
5	9	41	66
6	8	30	55
7	3	14.5	28.3
8	11	40	67
9	2	4	6
10	5	12	14
11	1	2	2
15	2	4	13
16	2	5	10



The results of the *in vivo* tests correlate perfectly with those of the *in vitro* tests. By way of example, microcapsules of Example 1 were injected intramuscularly into rats at a dose of 1.2 mg/kg. A plasma analysis revealed that the amount of triptorelin remained constantly above 0.1 ng/ml over a period of more than 90 days. The same studies conducted on microcapsules of Example 2 showed that the amount of testosterone remained constantly below 1 ng/ml over a period of more than 90 days. Furthermore, microcapsules of example 9 have been injected intramuscularly into rats at a dose of 1.2 mg/kg and a plasma analysis revealed that the amount of triptorelin remained constantly above 0.1 ng/ml over a period of more than 90 days.



The microimplants of Example 12 were tested *in vivo* as follows: a total dose of 3 mg of triptorelin was injected intramuscularly into 6 beagle dogs (weighing about 12 kg), into a muscle of the back paw of each of the animals. A plasma analysis revealed that the amount of triptorelin remained constantly above 0.1 ng/ml over a period of more than 90 days.

Where the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification, they are to be interpreted as specifying the presence of the stated features, integers, steps or components referred to, but not to preclude the presence or addition of one or more other feature, integer, step, component or group thereof.



CLAIMS

1. Composition in the form of microcapsules or implants comprising a biodegradable polymeric or copolymeric excipient or a mixture of such excipients and an active substance or a mixture of active substances, it being possible for said
5 microcapsules or said implants to release the active substance or the mixture of active substances over a prolonged period of up to three months or more according to an essentially monophasic release profile, said composition being characterized in that :
- either, when the composition is in the form of microcapsules:
- 10 • either the viscosity of said polymers or copolymers is comprised between 0.7 dl/g and 1.6 dl/g in CHCl_3 and the preparation process for said microcapsules does not comprise any stage of fusion of said microcapsules,
 • or the viscosity of said polymers or copolymers is comprised between 0.5 dl/g and 1.6 dl/g in CHCl_3 and said polymers or copolymers have an
15 hydrophilic character ;
- or, when the composition is in the form of implants, the viscosity of said polymers or copolymers is comprised between 0.5 dl/g and 1.6 dl/g in CHCl_3 .
2. Composition in the form of microcapsules or implants according to claim 1, characterized in that the biodegradable polymeric or copolymeric excipient has an
20 inherent viscosity at least equal to 0.9 dl/g in CHCl_3 .
3. Composition in the form of microcapsules or implants according to one of claims 1 or 2, characterized in that the biodegradable polymer is a copolymer of lactide and glycolide (PLGA).
4. Composition according to claim 3, characterized in that the acid number of
25 the polymer or copolymer is greater than 1 meq of KOH per gram of polymer or copolymer, and preferably greater than 1.2 or 1.5 meq of KOH per gram of polymer or copolymer.
5. Composition in the form of microcapsules or implants according to claim 3 or 4, characterized in that the PLGA is prepared from 70 to 80 % lactide and 20 to 30 %
30 glycolide.



6. Composition in the form of microcapsules or implants according to one of claims 1 to 5, characterized in that the biodegradable polymer is a copolymer of L-lactide and glycolide.
7. Composition in the form of microcapsules or implants comprising at least one biodegradable polymeric or copolymeric excipient or a mixture of such excipients, and at least one water-soluble active substance of high specific surface area.
8. Composition in the form of microcapsules or implants according to claim 7, characterized in that the specific surface area of the active substance is greater than $2 \text{ m}^2/\text{g}$ and preferably greater than $3 \text{ m}^2/\text{g}$.
9. Composition in the form of microcapsules or implants according to claim 8, characterized in that the specific surface area of the active substance is greater than $5 \text{ m}^2/\text{g}$ and preferably greater than $10 \text{ m}^2/\text{g}$.
10. Composition in the form of microcapsules or implants according to claim 9, characterized in that the specific surface area of the active substance is greater than $20 \text{ m}^2/\text{g}$ and preferably greater than $30 \text{ m}^2/\text{g}$.
11. Composition in the form of microcapsules or implants according to any one of claims 7 to 10, characterized in that the viscosity of the polymer or copolymer is comprised between 0.5 and 1.6 dl/g in CHCl_3 , et preferably comprised between 0.9 and 1.6 dl/g in CHCl_3 .
12. Composition in the form of microcapsules or implants according to any one of claims 7 to 11, characterized in that the polymer or copolymer presents an hydrophilic character, the acid number of the latter being greater than 1 meq KOH per gram of polymer or copolymer, and preferably greater than 1.2 or 1.5 meq KOH per gram of polymer or copolymer.
13. Composition in the form of microcapsules or implants according to any one of claims 7 to 12, characterized in that the polymer or copolymer is a PLGA, and preferably a PLGA prepared from 70 to 80 % lactide and 20 to 30 % glycolide.
14. Composition in the form of microcapsules or implants according to any one of claims 1 to 13, characterized in that the active substance is a protein or a peptide.



15. Composition in the form of microcapsules or implants according to one of the preceding claims, characterized in that the active substance is selected from the group consisting of the following substances: triptorelin or one of its salts, particularly triptorelin acetate, lanreotide or one of its salts, particularly lanreotide acetate, octreotide or one of its salts, particularly octreotide acetate or pamoate, a
5 compound with LH-RH activity, such as triptorelin, goserelin, leuprorelin, buserelin or their salts, an LH-RH antagonist, a GPIIb/IIIa antagonist, a compound with a similar activity to a GPIIb/IIIa antagonist, erythropoietin (EPO) or one of its analogues, the various types of interferon- α , interferon- β or - γ , somatostatin, a
10 somatostatin derivative, a somatostatin analogue, insulin, a growth hormone, a growth hormone releasing factor (GHRF), a growth hormone releasing peptide (GHRP), an epidermal growth factor (EGF), a melanocyte stimulating hormone (MSH), a thyrotropin releasing hormone (TRH) or one of its salts or derivatives, a
15 thyroid stimulating hormone (TSH), a luteinizing hormone (LH), a follicle stimulating hormone (FSH), a parathyroid hormone (PTH) or one of its derivatives, a lysozyme hydrochloride, a parathyroid hormone related peptide (PTHrp), an N-terminal peptide fragment (position 1-34) of human PTH hormone, vasopressin or one of its derivatives, oxytocin, calcitonin, a calcitonin derivative with a similar activity to that of calcitonin, a calcitonin gene related peptide (CGRP), glucagon, a
20 peptide similar to glucagon (GLP), gastrin, a gastrin releasing peptide (GRP), secretin, pancreaticozym, cholecystokinin, angiotensin, human placental lactogen, human chorionic gonadotropin (HCG), enkephalin, an enkephalin derivative, colony stimulating factor (CSF), endorphin, kytorphin, interleukins, for example interleukin-2, tuftsin, thymopoietin, thymostimulin, thymic humoral factor (THF),
25 thymic serum factor (TSF), a derivative of thymic serum factor (TSF), thymosin, thymic factor X, tumour necrosis factor (TNF), motilin, bombesin or one of its derivatives, prolactin, neurotensin, dynorphin, caerulein, substance P, urokinase, asparaginase, bradykinin, kallikrein, nerve growth factor, a blood clotting factor, polymixin B, colistin, gramicidin, bacitracin, a protein synthesis stimulating peptide,
30 an endothelin antagonist or one of its salts or derivatives, a vasoactive intestinal polypeptide (VIP), adrenocorticotrophic hormone (ACTH) or one of its fragments, a platelet derived growth factor (PDGF), a bone morphogenetic protein (BMP), a pituitary adenylate cyclase activating polypeptide (PACAP), neuropeptide Y (NPY), peptide YY (PYY), a gastric inhibitory polypeptide (GIP) and polynucleotides,
35 especially double-stranded RNAs.



16. Composition in the form of microcapsules or implants according to one of claims 1 to 15, characterized in that the active substance is chosen from the group consisting of triptorelin acetate, lanreotide acetate or octreotide acetate.

17. Process for the preparation of a water-soluble substance of high specific surface area, comprising the following steps:

- a lyophilization step comprising the rapid immersion of a dilute solution of said water-soluble substance in a medium whose temperature is below -50°C , and preferably below -70°C ; and
- optionally a grinding step, which preferably comprises ultrasonic grinding.

18. Process according to claim 17, characterized in that the dilute solution is a solution whose concentration is less than half the saturation concentration.

19. Process according to claim 17 or 18, characterized in that the water-soluble substance has a saturation concentration of at least 200 g/l and in that the dilute solution is a solution whose concentration is less than a quarter of the saturation concentration.

20. Process according to one of claims 17 to 19, characterized in that the rapid immersion is carried out after a step for micronization of the solution of active substance, said micronization step preferably consists in passing the solution through an atomizer.

21. Active substance, preferably a protein or a peptide, as obtained by a process according to one of claims 17 to 20.

22. Active substance according to claim 21, characterized in that it is selected from the group consisting of the following substances: triptorelin or one of its salts, particularly triptorelin acetate, lanreotide or one of its salts, particularly lanreotide acetate, octreotide or one of its salts, particularly octreotide acetate, a compound with LH-RH activity, such as triptorelin, goserelin, leuprorelin, buserelin or their salts, an LH-RH antagonist, a GPIIb/IIIa antagonist, a compound with a similar activity to a GPIIb/IIIa antagonist, erythropoietin (EPO) or one of its analogues, the various types of interferon- α , interferon- β or - γ , somatostatin, a somatostatin derivative, a somatostatin analogue, insulin, a growth hormone, a growth hormone releasing



- factor (GHRF), a growth hormone releasing peptide (GHRP), an epidermal growth factor (EGF), a melanocyte stimulating hormone (MSH), a thyrotropin releasing hormone (TRH) or one of its salts or derivatives, a thyroid stimulating hormone (TSH), a luteinizing hormone (LH), a follicle stimulating hormone (FSH), a
- 5 parathyroid hormone (PTH) or one of its derivatives, a parathyroid hormone related peptide (PTHrp), an N-terminal peptide fragment (position 1→34) of human PTH hormone, vasopressin or one of its derivatives, oxytocin, calcitonin, a calcitonin derivative with a similar activity to that of calcitonin, a calcitonin gene related peptide (CGRP), glucagon, a peptide similar to glucagon (GLP), gastrin, a gastrin
- 10 releasing peptide (GRP), secretin, pancreaticozymin, cholecystokinin, angiotensin, human placental lactogen, human chorionic gonadotropin (HCG), enkephalin, an enkephalin derivative, colony stimulating factor (CSF), endorphin, kyotorphin, interleukins, for example interleukin-2, tuftsin, thymopietin, thymostimulin, thymic humoral factor (THF), thymic serum factor (TSF), a derivative of thymic serum
- 15 factor (TSF), thymosin, thymic factor X, tumour necrosis factor (TNF), motilin, bombesin or one of its derivatives, prolactin, neurotensin, dynorphin, caerulein, substance P, urokinase, asparaginase, bradykinin, kallikrein, nerve growth factor, a blood clotting factor, polymixin B, colistin, gramicidin, bacitracin, a protein synthesis stimulating peptide, an endothelin antagonist or one of its salts or
- 20 derivatives, a vasoactive intestinal polypeptide (VIP), adrenocorticotrophic hormone (ACTH) or one of its fragments, a platelet derived growth factor (PDGF), a bone morphogenetic protein (BMP), a pituitary adenylate cyclase activating polypeptide (PACAP), neuropeptide Y (NPY), peptide YY (PYY), a gastric inhibitory polypeptide (GIP) and polynucleotides, especially double-stranded RNAs.
- 25 23. Triptorelin acetate, lanreotide acetate or octreotide acetate as obtained by a process according to one of claims 17 to 20.
24. Double-stranded RNA, preferably polyadenylic acid complexed with polyuridylic acid, as obtained by a process according to one of claims 17 to 20.



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